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Helicobacter pylori seroprevalence in patients with chronic obstructive pulmonary disease

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Summary An increased seroprevalence of *Helicobacter pylori* (*H. pylori*) and especially of the high virulent cytotoxin-associated gene-A (CagA) positive strains has been found in several extragastroduodenal pathologies, characterized by activation of inflammatory mediators. Moreover, it has been reported that the risk of chronic bronchitis may be increased in *H. pylori* infected patients. The aim of the present study was to assess the seroprevalence of *H. pylori* and in particular of CagA-positive virulent strains in patients with chronic obstructive pulmonary disease (COPD). We evaluated 126 COPD patients (88 males and 38 females, aged 61.3±8.1 years) and 126, age and sex-matched, control subjects. All subjects enrolled underwent an enzyme-linked immunosorbent assay (ELISA) IgG serologic test for *H. pylori* and CagA protein. The prevalence of *H. pylori* infection in patients and controls was 77.8% and 54.7%, respectively ($P<0.001$) and that of CagA-positive *H. pylori* infection was 53.9% and 29.3%, respectively ($P<0.001$). Moreover, COPD patients had a significantly increased mean serum concentration of both anti-*H. pylori* IgG (118.3±24.4 vs. 61.9±12.9 U/ml, $P<0.001$) and anti-CagA IgG antibodies (33.8±3.4 vs. 19.0±1.5 U/ml, $P<0.001$). Finally, no statistically significant difference, as regards the spirometric values, was detected between *H. pylori* infected COPD patients and uninfected ones. In conclusion, *H. pylori* infection may be associated with COPD. Further studies should be undertaken to clarify the potential underlying pathogenetic mechanisms.

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Introduction

Helicobacter pylori (*H. pylori*) is a slow-growing, microaerophilic, gram-negative bacterium which colonizes gastric mucosa and elicits both inflammatory and immune lifelong responses, with release of various bacterial and host-dependent cytotoxic substances.[1] This bacterium seems to have a causative role in the development of chronic gastritis,[2] peptic ulcer disease,[3] low-grade B-cell lymphoma of gastric mucosa-associated-lymphoid-tissue (MALT-lymphoma)[4] and gastric cancer.[5] Recent studies suggest that *H. pylori* infection might, also, be associated with several extragastroduodenal pathologies characterized by activation of inflammatory mediators and/or induction of autoimmunity.[6–8] Therefore, increased *H. pylori* seroprevalence has been found in ischemic heart disease,[9] rosacea[10] and active bronchiectasis.[11] In patients with ischemic heart disease, an extremely high prevalence of the cytotoxin-associated gene-A (CagA) positive virulent strain of *H. pylori* has also been reported.[12] The CagA-positive are those strains that induce increased local and systemic, humoral and cellular inflammatory response.[13]

It is well known that the prevalence of chronic obstructive pulmonary disease (COPD) in peptic ulcer patients is increased two-to-three fold, compared with findings in ulcer-free controls.[14–16] The major factor underlying this association seems to be the impact of cigarette smoking on both diseases. However, in 1998, a pilot study in a small number of Italian patients showed that *H. pylori* infection, per se, might be related to an high risk of developing chronic bronchitis.[17] More recently, an epidemiological study in Danish adults suggested that chronic bronchitis might be more prevalent in *H. pylori* IgG-seropositive women than in uninfected ones.[18] These observations prompted us to perform a case-control study in a cohort of Greek patients with chronic bronchitis. We found extremely high *H. pylori* seropositivity.[19] However, the prevalence of *H. pylori* and especially of CagA-positive strains (that induce increased inflammatory response and have been associated with other extraintestinal disorders), in COPD patients remains still unknown.

Therefore, the aim of the present study was to assess the seroprevalence of *H. pylori* and in particular of CagA-positive virulent strains in a cohort of COPD patients and control subjects (outpatients with a variety of respiratory diseases). Moreover, we evaluated the association between *H. pylori* serological status and spirometric values in patients with COPD.

Methods

Patients selection

The present study was conducted at the 9th Department of Pulmonary Medicine, ''Sotiria'' Chest Diseases Hospital (Athens, Greece). The local ethics committee approved the study and written informed consent was obtained from each participant. Following a predefined protocol, between June 1, 2002 and October 31, 2003, 178 consecutive patients with COPD, diagnosed according to the Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD) guidelines, were recruited from the outpatient clinics. Briefly, COPD was diagnosed as ''the presence of a postbronchodilator FEV_1 $<80\%$ of the predicted value in combination with an FEV_1/FVC $<70\%$ in any patient who has symptoms of cough, sputum production, or dyspnea and/or a history of exposure to risk factors for the disease''.[20] Exclusion criteria were: (i) an exacerbation of COPD in the preceding month, as in those cases pulmonary function does not represent baseline levels, (ii) prior *H. pylori* eradication therapy, (iii) consumption of acid-suppressive drugs or antibiotics in the preceding 6 months and (iv) a history of vagotomy or operation on the upper gastrointestinal tract. A total of 52 patients were excluded. Therefore, 126 patients were eligible for analysis.

Control subjects selection

Controls were selected randomly from 204 consecutive subjects with other pulmonary disorders attending the outpatient clinics during the period of study (bronchial asthma, respiratory infections, lung cancer and sarcoidosis). Briefly, bronchial asthma was diagnosed as the ''presence of symptoms of episodic wheezing, cough and shortness of breath responding to bronchodilators and reversible airflow obstruction documented in at least one previous pulmonary function study'' Exclusion criteria for controls were: (i) a known history of COPD, (ii) a known history of gastrointestinal tract pathology including *H. pylori* infection and (iii) consumption of acid-suppressive drugs or antibiotics in the preceding 6 months. Finally, we selected 126 controls from among 204 subjects (60 with respiratory infections, 38 with lung cancer, 22 with asthma and 6 with sarcoidosis). Control subjects were matched with the COPD patients for sex, age (within 2 years) and socioeconomic status. Social class classification was based on the current

occupation according to the classification system of the United Kingdom Registrar General. Assignment to class group is determined as follows: social class I: skilled professionals, social class II: intermediate manual workers, social class III: skilled manual workers, social class IV: partly skilled manual workers and social class V: unskilled manual workers. All unemployed housewives were classified according to their husbands' occupation.

Lung function—COPD severity

In all COPD patients a complete medical history was taken and a physical examination was performed. Moreover, in all cases postbronchodilation spirometric values (FEV_1, FVC, FEV_1/FVC) were measured. The best value of three maneuvers was expressed as a percentage of the predicted value. Finally, classification of COPD severity was performed according to GOLD guidelines.[20] Briefly, three stages of COPD according to disease severity were recognized:

(I) Stage I (mild COPD) was characterized by mild airflow limitation (FEV_1/FVC $<70\%$ and $FEV_1 > 80\%$ predicted).
(II) Stage II (moderate COPD) was characterized by worsening airflow limitation ($30\% < FEV_1 < 80\%$ predicted) and
(III) Stage III (severe COPD) was characterized by severe airflow limitation ($FEV_1 < 30\%$ predicted) or the presence of respiratory failure or clinical signs of right heart failure.

Serological parameters

All subjects enrolled (COPD patients and controls) underwent an enzyme-linked immunosorbent assay (ELISA) IgG serologic test for *H. pylori* and CagA protein detection (HEL-P test, Park Co, Athens, Greece), in accordance with the manufacturer's guidelines. The specificity and sensitivity of the serology test, validated in our local population, were 95% and 85%, respectively.

All results were analyzed simultaneously by technicians who were unaware of whether the sample belonged to cases or controls. A positive, borderline and negative result was assigned when the concentration of IgG antibodies against *H. pylori* was greater than 25, between 20 and 25 and less than 20 U/ml, respectively. Moreover, when the concentration of IgG CagA antibodies was greater than 7.5 U/ml, between 5.5 and 7.5 U/ml and less than 5.5 U/ml the result was considered as positive, borderline and negative, respectively. Borderline results were omitted from further analysis.

Statistical analysis

Results are expressed as mean $\pm$ one standard deviation ($\pm$ SD). Significance of difference between groups was assessed by unpaired Student's *t*-test for continuous variables and χ^2-test for proportions. The statistical analysis was performed using the SPSS program (SPSS Inc, IL, USA) and *P*-values were two-tailed analyzed. *P*-values of less than 0.05 were considered statistically significant.

Results

The demographic data of both patients and controls are shown in Table 1.

There was no significant difference in age or sex distribution between the two groups. Table 1 shows also the spirometric values of patients with COPD. Among the COPD patients, 35 (27.7%) had mild disease (Stage I according to GOLD classification), 68 (53.9%) had moderate disease (Stage II) and 23 (18.4%) had severe COPD (Stage III).

Table 2 shows the analysis of the serological parameters. Both anti-*H. pylori* IgG seropositivity and anti-CagA IgG seropositivity were significantly higher in COPD patients than in control subjects. Moreover, COPD patients had a significantly increased mean serum concentration of both anti-*H. pylori* IgG and anti-CagA IgG antibodies.

The distribution of COPD patients according to COPD severity was as follows: (i) Stage I (mild COPD): 35 patients (27.7%), (ii) Stage II (moderate COPD): 68 patients (53.9%) and (iii) Stage III (severe COPD): 23 patients (18.4%). The spirometric values of COPD patients in relation with *H. pylori* infection

Table 1 Demographic data of COPD patients and controls and spirometric values of COPD patients.

Parameters	COPD patients ($n = 126$)	Controls ($n = 126$)	*P*-value
Age (yr)	61.3 ± 8.1	59.0 ± 7.3	ns*
Male sex (%)	69.8	69.8	ns*
FEV_1†	61.9 ± 18.5		
FEV_1/FVC†	63.2 ± 4.8		

*Not significant.
†Expressed as percentages of the predicted values.

Table 2 Serological parameters in COPD patients and control subjects.

Parameters	COPD patients ($n = 126$)	Control subjects ($n = 126$)	*P*-value
Anti-*H. pylori* IgG seropositivity (%)	77.8	54.7	<0.001
Anti-*H. pylori* IgG level (U/ml)	118.3 ± 24.4	61.9 ± 12.9	<0.001
Anti-CagA IgG seropositivity (%)	53.9	29.3	<0.001
Anti-CagA IgG level (U/ml)	33.8 ± 3.4	19.0 ± 1.5	<0.001

Table 3 The spirometric values of COPD patients in relation with *H. pylori* infection.

Parameters	*H. pylori* positive ($n = 98$)	*H. pylori* negative ($n = 28$)	*P*-value
FEV_1*	61.5 ± 18.9	63.5 ± 17.9	ns†
FEV_1/FVC*	63.0 ± 4.9	63.9 ± 4.6	ns†

*Expressed as percentages of the predicted values.
†Not significant.

are shown in Table 3. No statistically significant difference, as regards the values, was detected between *H. pylori* infected COPD patients and uninfected ones. Finally, the spirometric values did not differ significantly between COPD patients infected with CagA-positive strains (FEV_1: 59.1 ± 19.7, FEV_1/FVC: 65.4 ± 16.6) and those infected with CagA-negative strains (FEV_1: 62.4 ± 5.1, FEV_1/FVC: 64.3 ± 4.3, *P*: not significant).

Discussion

Data in the literature on the relationship between *H. pylori* infection and chronic obstructive pulmonary disease (COPD) are poor. COPD had been associated with gastroduodenal ulcer many years before the identification of *H. pylori* infection as a cause of peptic ulcer disease. Three epidemiological studies, carried out between 1968 and 1986, showed that the prevalence of COPD in peptic ulcer patients was increased two-to-three fold compared with that in ulcer-free controls.[14–16] Moreover, a follow-up study demonstrated that COPD was a major cause of death among patients with peptic ulcer disease.[21] The reported association between these two diseases was, originally, attributed to the known role of cigarette smoking as an independent factor in both ulcerogenesis and development of COPD.[22] However, two recent studies showed that a subpopulation of COPD patients, those with chronic bronchitis, might also have an increased prevalence of *H. pylori* infection.[17,19]

The present study is the first focused on the seroprevalence of *H. pylori* and in particular of CagA-positive virulent strains in a large population of patients with COPD. Patients who were at risk for COPD (STAGE 0 according to GOLD) were not included in our study as those patients might have only limited differences as regards lung function with controls, a fact that might represent a potential study limitation. According to our results, both anti-*H. pylori* and anti-CagA seropositivity were significantly higher in COPD patients than in control subjects. The socioeconomic status, which is related with both *H. Pylori* infection and risk of COPD, is similar in the two groups. Tobacco use could be another confounding factor. Cigarette smoking is the most important etiologic factor of COPD. However, data on the relationship between *H. pylori* infection and smoking habits are controversial. The prevalence of *H. pylori* infection in smokers has been variously reported as low,[23] normal,[24] and high.[25] In the present study, we did not match patients and control subjects for smoking habits. As the relation between smoking and *H. pylori* colonization of gastric mucosa has not been clarified yet, the possible impact of cigarette smoking on both COPD and *H. pylori* infection should be regarded as a potential study limitation.

The selection of control subjects should be considered as another study limitation. It has been suggested, in a few studies, that *H. pylori* might be increased in a variety of respiratory disorders including lung cancer, bronchiectasis and tuberculosis.[8] Therefore, the selection of patients with respiratory diseases as controls may have reduced

the difference in positive seroprevalence between the groups. However, the existed difference could not be attributed to this selection, as a low *H. pylori* seroprevalence in respiratory diseases has not been reported yet.[8]

The present study has not focused on the potential pathogenetic mechanisms underlying the association between *H. pylori* infection and COPD. This association might reflect either susceptibility induced by common factors or a kind of causal relationship between these diseases. As far as we know, there are no common factors implicated in the susceptibility to both COPD and *H. pylori* infection. However, we cannot rule out this possibility, as the predisposing conditions to *H. pylori* infection have not been clarified yet.

With regard to the potential aetio-pathogenetic role of *H. pylori* infection in COPD, the chronic activation of inflammatory mediators induced by *H. pylori* infection might lead to the development of COPD. The increased prevalence of CagA positive strains in our study population further supports this hypothesis. It is well known that these virulent strains stimulate the release of a variety of proinflammatory cytokines, including Interleukin-1 (IL-1), IL-8 and tumour necrosis factor-alpha.[26,27] Moreover, eradication of *H. pylori* leads to normalization of serum cytokines levels.[28] These cytokines are also thought to be involved in the pathogenesis of COPD.[29–31] Therefore, *H. pylori* infection in general and CagA-positive strains in particular might play a proinflammatory role and co-trigger COPD with other more specific environmental, genetic and unknown factors. The lack of association between spirometric values and *H. pylori* infection, reported in our study, suggest that *H. pylori* might have a minor role in the further progression of the disease.

Another potential pathogenetic mechanism could be the spilling or inhalation of *H. pylori* or its exotoxins into the respiratory tract, which also might lead to a chronic airway inflammation such as COPD. However, as far as we know, neither identification of *H. pylori* species in human bronchial tissue, nor isolation of *H. pylori* from bronchoalveolar lavage (BAL) fluid has been achieved yet.[32]

In conclusion, the present study suggests that patients with COPD have an increased seroprevalence of *H. pylori* infection. Our results must be confirmed in a larger number of patients. Further studies should be undertaken to clarify the pathogenetic mechanisms underlying the possible association between these diseases and the effect of *H. pylori* eradication on the natural history of COPD.

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